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Heat tolerance indicators in Pakistani wheat (*Triticum aestivum* L.) genotypes

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Abstract – The effect of high temperature stress on six wheat cultivars exposed to 35–40 °C for 3 h each day for five consecutive days was examined. High temperature significantly affected total proline, soluble protein content, membrane stability index (MSI), yield, and various yield components, and had a direct effect on growth and other physiological attributes of wheat at anthesis and the milky seed stages. The wheat cultivar AS-2002 achieved better osmotic adjustment by accumulating more leaf proline. Higher MSI was also observed in AS-2002, as well as Inqalab-91. The anthesis growth stage was found to be more sensitive to heat stress than seed development at the milky stage. Overall heat stress reduced yield 75% at anthesis and 40% at the milky stage. AS-2002 performed better on the basis of yield and yield components. Seed weight per spike was highest in AS-2002, and lowest in SH-2002. The cumulative response of AS-2002 was better on the basis of physiological and yield attributes. In addition to yield, plant breeders should also include proline and MSI as selection parameter in the breeding program for development of heat tolerant wheat cultivars. Most of the evaluated wheat cultivars/lines were developed for cultivation in the rainfed areas of Pakistan.

Keywords: anthesis, grain yield, heat stress, membrane stability index, milky seed stage, proline, soluble proteins, *Triticum aestivum* L., wheat

Introduction

High temperature is a major problem in field cropping systems world-wide, with unexpected spatial and temporal variations causing reduced plant growth and productivity (Parent et al. 2010). It has been estimated that a rise in temperature of just 1 °C in wheat during the growing season reduces wheat yields by about 3–10% (You et al. 2009). Wheat is the

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major staple food crop of Pakistan, where the estimated per capita consumption is about 124 kg year^{-1}, among highest in the world. In order to meet the demand for food in Pakistan, an increase in wheat production of at least 4% is required to keep up with population growth. In Pakistan, wheat yields are especially sensitive to heat stress during the flowering to seed maturing stages. During this period, heat stress shortens the growth cycle and forces premature ripening, reduces the number of grains per spike, lowers grain weight, and ultimately results in grain yield and quality deterioration (Khan et al. 2007, Wahid et al. 2007, Din et al. 2010). Therefore, an urgent need exists to develop wheat cultivars which are better able to withstand heat stress during later growth stages, or else mature earlier to escape the heat stress typically occurring later in the growing season.

Understanding the means by which crop plants like wheat can tolerate heat would lay the foundation for work to develop plants with improved heat tolerance. One of the most common responses of crop plants to high temperature stress is an increase in proline accumulation (Ahmed and Hasan 2011). Under high temperature, free proline is involved in osmotic adjustment to protect pollen and plant enzymes from heat injury, and also provides a source of nitrogen and other metabolites (Verslues and Sharma 2010). Accumulation of proline has been shown to occur under heat stress in arabidopsis (Wei-Tao et al. 2011), cotton (Ronde et al. 2001) and wheat (Hasan et al. 2007), and genotypic variation in proline accumulation have been reported for these species. Under high temperature certain heat shock genes are triggered, resulting in the synthesis of heat shock proteins, whereas other soluble and insoluble proteins have also been shown to exhibit changes in abundance under high temperature stress (Simmonds 1995, He et al. 2005).

High temperature can cause a loss of membrane integrity, damage to primary photosynthetic processes, and changes in lipid composition, and protein denaturation (Wahid et al. 2007). Membrane thermal stability due to heat stress, typically measured as ion leakage from the cell, has been used for screening wheat germplasm for thermal tolerance (Yildirim et al. 2009). Blum et al. (2001) showed a higher yield in spring wheat lines having greater membrane-thermostability in flag leaves at anthesis. The study presented here was conducted to explore the physiological basis for heat stress tolerance during later growth stages in wheat, and recommend reliable screening strategies for heat tolerance that can be utilized in wheat breeding programs for Pakistan and elsewhere. Investigated genotypes were developed for cultivation in rainfed areas of Pakistan so they could be further used as parent material for development of heat tolerant wheat cultivars in the domestic wheat breeding program.

Materials and methods

Six wheat genotypes AS-2002, Inqalab-91, Punjab-96, NR-234, Wafaq-2001 and SH-2002 obtained from Wheat Program, Crop Sciences Institute, National Agricultural Research Centre (NARC), Pakistan, were used in the study. Wheat plants were grown in pots (30 × 40 cm size) containing 10 kg sandy loam soil in a greenhouse under natural daylight at NARC, Islamabad (latitude 33.38°N, longitude 73.00°E) during the winter/spring with average day/night temperature 30 ± 8 °C and 13 ± 5 °C, respectively. A recommended dose of NPK (120-100-60 kg ha^{-1}) was applied as urea, diammonium phosphate and potassium sulphate. The pots were arranged in factorial, randomized, complete block design. Plants

were subjected to specific heat stress treatments immediately at anthesis (80 days after sowing) and at milky growth stages (120 days after sowing). At anthesis when the first anther extrusion occurred, pots (three replications) each containing three plants were moved to a greenhouse where temperature was maintained at 35–40 °C and 14/10 h day/night, 50–70% relative humidity, and illumination of 335 μmol m^{-2} s^{-2}. After high temperature treatment for 3 h daily for five consecutive days, pots were moved back to normal temperature (average day/night temperature 30 ± 8 °C and 13 ± 5 °C) conditions in open greenhouse atmosphere. These heat treatments were also applied to the second set of potted plants during the milky growth stage.

After daily 3-h heat stress treatments on five consecutive days, flag leaf from the control and stressed plants was sampled for analysis of proline, soluble protein, and membrane stability index (MSI). Total proline was determined using the method of Bates et al. (1973). Fresh plant tissue was extracted with 3% aqueous 5-sulfosalicylic acid and the filtrate was reacted with glacial acetic acid and ninhydrin solution at 100 °C for 1 h. The reaction mixture was extracted with toluene and the absorbance of the chromophore contain toluene was read at 520 nm.

The leaf MSI was determined according to Sairam et al. (2002). Leaf strips (0.2 g) of uniform size were taken in a test tube containing 10 mL double distilled water in two sets. Test tubes in one set were kept at 40 °C in a water bath for 30 min and electrical conductivity of the water containing the sample was measured (C_1) using a conductivity bridge. Test tubes of the other set were incubated at 100 °C in boiling water for 15 min and their electrical conductivity was measured as above (C_2). MSI was calculated using formula as below:

$$MSI = [1 - (C_1 / C_2)] \times 100$$

Soluble protein content was determined according to Bradford (1976). Fresh plant tissue was extracted with 0.15 M NaCl and the filtrate was reacted with Bradford reagent (Bio-Rad protein assay dye reagent). Protein concentration of the sample was calculated using the calibration curve of bovine serum albumin and expressed on a fresh weight basis. At physiological maturity, grain yield per plant, florets per spike, number of seeds, seed weight per spike, and number of sterile florets per spike of both the heat stressed and the control plants were recorded.

The analysis of variance of the data for each attribute was carried out using Minitab version 13.1. The mean values were compared with Duncan's multiple range (DMR) test at significant differences (ANOVA) ($P < 0.05$) following Snedecor and Cochran 1980.

Results

Impact of heat on proline, total protein, and the membrane stability index (MSI)

Heat stress imposed at anthesis and milky growth stages significantly increased proline concentration in leaves of all the wheat genotypes in comparison to their control values. At anthesis, the highest increase was recorded in AS-2002 (89%) and lowest in Punjab-96 (76%) (Fig. 1a). At the milky stage, the trend of relative increase in proline concentration was lower in AS-2002 (76%) followed by Inqalab-91 (77%) and even higher in the rest of the genotypes (Fig. 1b).

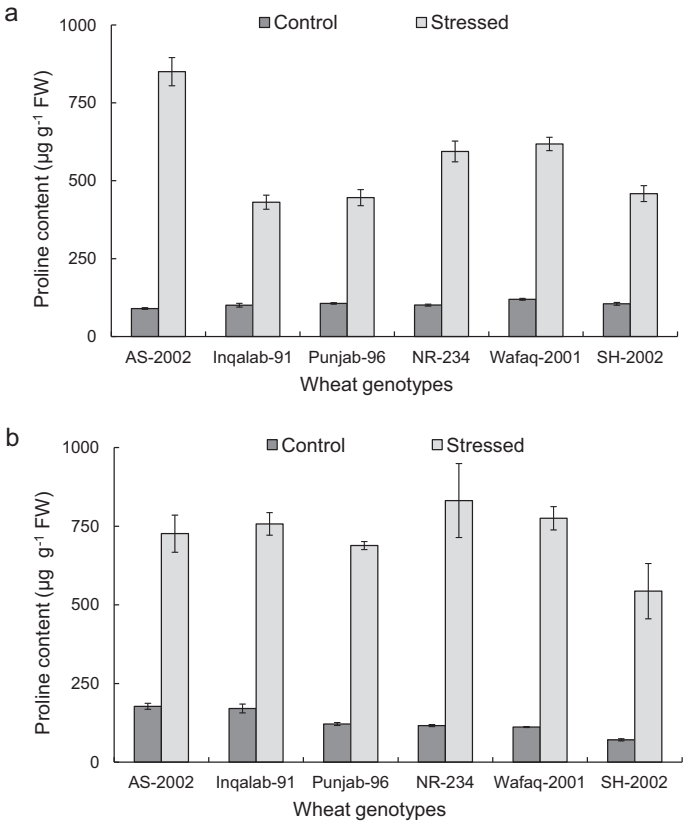

Fig. 1. Effect of heat stress on the leaf proline concentration (µg g^{-1} fresh weight): **(a)** at anthesis, and **(b)** milky growth stages of wheat genotypes. The vertical bars indicate standard error (± SE) of mean (n = 3). All means are significantly different at $p < 0.05$.

Under heat stress, increases in soluble protein content were recorded at both anthesis and milky growth stages (Figs. 2a, b). The increase in soluble protein concentration was comparable in all the genotypes, and there was only slight, non-significant change in leaf protein content.

High temperature decreased the MSI at both anthesis and milky growth stages in all wheat genotypes tested. At anthesis, the decrease in MSI was greater in SH-2002 (23%) and least in AS-2002 (11%) and Inqalab-91 (15%). A similar trend toward decreased MSI was exhibited by all the genotypes during the milky growth stage (Fig. 3).

Impact of heat on grain yield, and the number of florets and seeds per spike

The heat treatment significantly decreased the grain yield per plant in all the tested wheat genotypes, at both anthesis and milky growth stages, and there was significant variation in grain yield within the genotypes, both at anthesis and milky growth stages (Tab. 1). Anthesis growth was more sensitive to heat stress, as heat treatments during this stage reduced yield by 75%, as compared to milky stage treatments that decreased yield by only

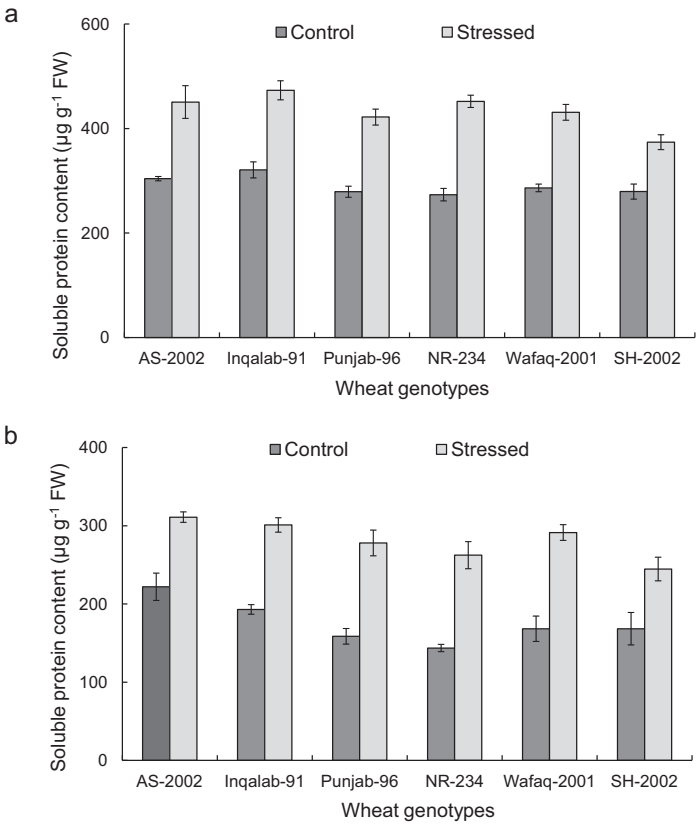

Fig. 2. Effect of heat stress on the soluble leaf protein concentration (µg g^{-1} fresh weight): **(a)** at anthesis, and **(b)** milky growth stages of wheat genotypes. The vertical bars indicate standard error (± SE) of mean (n = 3). All means are significantly different at $p < 0.05$.

40%, relative to non-treated controls. At anthesis, the magnitude of decrease in grain yield was least in AS-2002 (62.9%), whereas the decrease in grain yield in other genotypes was almost equal. At the milky stage, the smallest decrease in grain yield occurred for AS-2002 (16.2%), whereas the greatest decrease occurred for SH-2002 (57.5%). Similarly, heat treatment significantly decreased seed weight per spike at anthesis growth stage, in all the tested wheat genotypes except cv. AS-2002 (Tab. 2). The highest seed weight per spike after stress occurred with Inqalab-91 (1.06 g), whereas the lowest seed weight after stress treatment was found in NR-234 (0.54 g). At milky stage, seed weight decreased for all the genotypes, and their responses to the heat treatments were similar. However this effect was not statistically significant among the tested wheat genotypes.

The number of florets per spike decreased as a result of heat treatment for all the tested genotypes; however the effect was not statistically significant. The decrease in number of florets was greater at anthesis and least at milky stage (Tab. 3). Heat stress significantly increased the number of sterile florets per spike in all the tested wheat genotypes at the anthesis growth stage but not at the milky stage (Tab. 4). The highest and significant number of

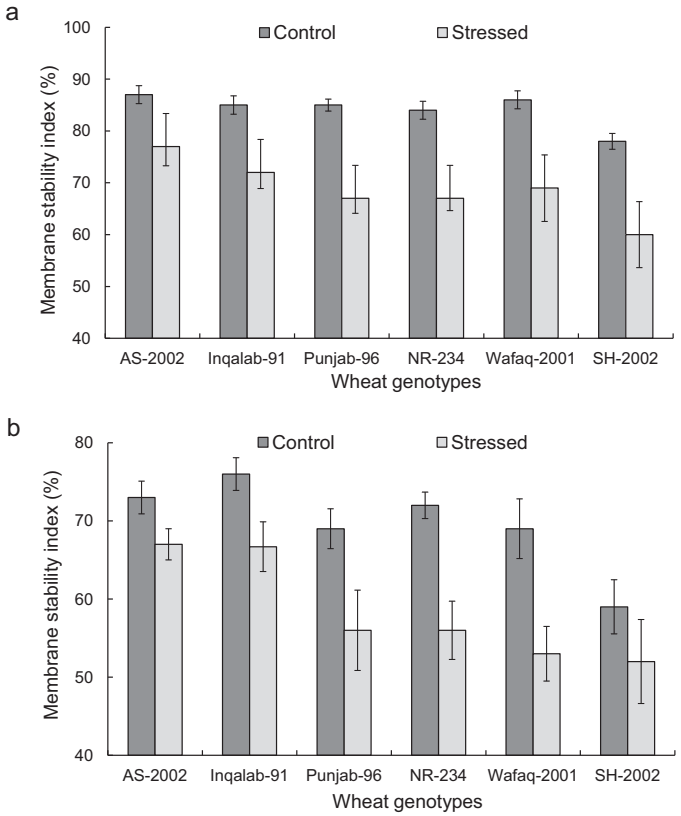

Fig. 3. Effect of heat stress on the leaf membrane stability index (%): **(a)** at anthesis, and **(b)** milky growth stages of wheat genotypes. The vertical bars indicate standard error (± SE) of mean (n = 3). All means are significantly different at $p < 0.05$.

Tab. 1. Effect of heat stress applied at anthesis and milky growth stages on the grain yield per plant (g plant^{-1}) of wheat genotypes. Values in columns having the same letter are not significantly different at $p > 0.05$, Duncan's multiple range test. Least significant difference (LSD) value (0.05) for variety × treatment interaction (V × T) = 1.535 at anthesis, LSD value (0.05) for variety × treatment interaction (V × T) = 1.674 at milky stage. The data in parentheses indicate percent decrease in grain yield per plant (g plant^{-1}) of wheat genotypes at anthesis and milky growth stages in comparison to their control values.

Genotypes	Anthesis stage		Milky stage	
	Control	Heat stress	Control	Heat stress
AS-2002	19.36±1.13 b	7.18±0.24 d (62.9)	19.36±1.13 b	16.23± 0.58 d (16.2)
Inqalab-91	16.40±0.55 c	5.00±0.05 e (69.5)	16.40±0.55 cd	10.56± 0.33 f (35.9)
Punjab-96	21.13±0.84 a	4.59±0.05 e (78.3)	21.13±0.84 a	14.26± 0.23 e (32.5)
NR-234	22.20±0.86 a	4.42±0.01 e (80.0)	22.20±0.86 a	13.73± 0.26 e (38.2)
Wafaq-2001	22.73±0.47 a	4.43±0.02 e (80.5)	22.73±0.47 a	9.83±0.15 f (56.8)
SH-2002	18.00±0.15 b	4.62±0.04 e (74.3)	18.00±0.15 bc	7.66±0.33 g (57.4)
Means	19.97	5.04 (75%)	19.97	12.05 (40%)

Tab. 2. Effect of heat stress applied at anthesis and milky growth stages on the seed weight per spike (g spike^{-1}) of wheat genotypes. Values in columns having the same letter are not significantly different at $p > 0.05$, Duncan's multiple range test. There were non-significant statistical differences for milky stage. Least significant difference (LSD) value (0.05) for variety × treatment interaction (V × T) = 0.316 at anthesis, LSD value (0.05) for variety × treatment interaction (V × T) = 0.801 at milky stage.

Genotypes	Anthesis stage		Milky stage	
	Control	Heat stress	Control	Heat stress
AS-2002	1.99±0.15 d	1.02±0.10 d	1.99±0.15	1.77±0.10
Inqalab-91	2.09±0.20 b	1.06±0.04 d	2.09±0.20	1.41±0.28
Punjab-96	2.46±0.11 a	0.83±0.18 de	2.46±0.11	1.12±0.04
NR-234	2.09±0.07 b	0.54±0.009 e	2.09±0.07	1.28±0.15
Wafaq-2001	1.93±0.07 bc	0.75±0.11 de	1.93±0.07	1.25±0.29
SH-2002	1.70±0.14 c	0.63±0.05 e	1.70±0.14	1.22±0.15

Tab. 3. Effect of heat stress applied at anthesis and milky growth stages on the number of florets per spike of wheat genotypes. There were non-significant statistical differences for anthesis and milky stage. Least significant difference (LSD) value (0.05) for variety × treatment interaction (V × T) = 10.28 at anthesis, LSD value (0.05) for variety × treatment interaction (V × T) = 8.312 at milky stage.

Genotypes	Anthesis stage		Milky stage	
	Control	Heat stress	Control	Heat stress
AS-2002	54.66±0.75	49.33±0.88	54.66±0.75	46.00±1.17
Inqalab-91	64.66±1.53	57.66±1.20	64.66±1.53	64.00±3.17
Punjab-96	46.66±0.75	32.33±1.85	46.66±0.75	54.30±3.33
NR-234	57.66±1.53	50.00±1.45	57.66±1.53	57.00±2.45
Wafaq-2001	58.66±0.75	44.00±1.60	58.66±0.75	49.00±1.05
SH-2002	55.66±0.96	42.00±2.08	55.66±0.96	46.00±1.97

Tab. 4. Effect of heat stress applied at anthesis and milky growth stages on the number of sterile florets per spike of wheat genotypes. Values in columns having the same letter are not significantly different at $p > 0.05$, Duncan's multiple range test. There were non-significant statistical differences for milky stage. Least significant difference (LSD) value (0.05) for variety × treatment interaction (V × T) = 4.193 at anthesis, and LSD value (0.05) for variety × treatment interaction (V × T) = 6.649 at milky stage.

Genotypes	Anthesis stage		Milky stage	
	Control	Heat stress	Control	Heat stress
AS-2002	8.66±1.20 d	24.33±1.20 b	8.66±1.20	11.00±0.61
Inqalab-91	8.66±0.66 d	33.33±1.20 a	8.66±0.66	13.30±0.55
Punjab-96	7.66±0.29 d	26.00±1.52 b	7.66±0.29	7.00±0.96
NR-234	4.33±0.24 d	15.33±1.85 c	4.33±0.24	8.00±0.22
Wafaq-2001	4.66±0.41 d	23.66±1.45 b	4.66±0.41	9.23±0.90
SH-2002	6.00±1.00 d	17.33±2.72 c	6.00±1.00	11.20±0.77
Means	6.67	23.32	6.67	9.95

sterile florets was recorded in Inqalab-91 (33.33) and Punjab-96 (26.0), and least in NR-234 (15.33) at anthesis stage. At anthesis growth stage, the number of seeds per spike also decreased significantly in all the tested wheat genotypes, except cv. Punjab-96 (14.8) (Tab. 5). Significant variations were observed among the genotypes. At anthesis stage, the quantity of decrease in number of seeds per spike was greater in NR-234 (41.7%) followed by SH-2002 (38.8%). At milky stage all the tested genotypes exhibited similar trends of floret number and sterility as a result of applied heat stress.

Tab. 5. Effect of heat stress applied at anthesis and milky growth stages on the number of seeds per spike of wheat genotypes. Values in columns having the same letter are not significantly different at $p > 0.05$, Duncan's multiple range test. There were non-significant statistical differences for milky stage. Least significant difference (LSD) value (0.05) for variety × treatment interaction (V × T) = 7.432 at anthesis, LSD value (0.05) for variety × treatment interaction (V × T) = 5.715 at milky stage. The data in parentheses indicate percent decrease in number of seeds spike^{-1} of wheat genotypes at anthesis and milky growth stages in comparison to their control values.

Genotypes	Anthesis stage		Milky stage	
	Control	Heat stress	Control	Heat stress
AS-2002	47.5±2.2 bcd	40.3±1.2 de (15.2)	47.5±2.2	44.7±0.9 (5.8)
Inqalab-91	64.0±1.2 a	51.7±0.6 bc (19.2)	64.0±1.2	59.3±3.1 (7.3)
Punjab-96	51.3±0.9 bc	43.7±0.3 cd (14.8)	51.3±0.9	50.7±2.7 (1.1)
NR-234	52.7±1.6 b	30.7±0.6 f (41.7)	52.7±1.6	48.7±2.3 (7.6)
Wafaq-2001	50.7±0.4 bc	33.0±0.7 ef (34.9)	50.7±0.4	47.4±1.1 (6.5)
SH-2002	49.0±1.2 bc	30.0±0.7 f (38.8)	49.0±1.2	45.7±1.3 (6.7)
Means	52.53	38.23 (27%)	52.53	49.42 (6%)

Discussion

In this study, heat stress imposed after anthesis and the milky stages resulted in changes in physiological attributes such as proline content, total soluble protein, MSI, yield parameters. Susceptibility to high temperatures may vary with the stage of plant development, but all vegetative and reproductive stages are affected by heat stress to some extent (Wahid et al. 2007). Heat stress induced modifications in plants may be observed as changes in specific physiological processes, or in varying effects on development, and these responses may vary from one growth stage to another. In the present study, heat stress application during reproductive stages, at either anthesis or the milky seed development stage, increased significantly the proline concentration in flag leaves of all the wheat genotypes examined. At anthesis stage, the increase was greater in AS-2002 and lowest in SH-2002, whereas at the milky stage, the increase was lower in AS-2002 and Inqalab-2001, and higher in rest of the genotypes. One of the most common responses of many plant species exposed to abiotic stresses is the accumulation of compatible organic solutes such as proline. Proline has been suggested to play a protective role in plants acting as a cellular osmotic regulator between cytoplasm and vacuole, and by its ability to detoxify reactive oxygen species (ROS) and thereby protecting membrane integrity and stabilizing antioxidant enzymes (Ashraf and Foolad 2007). Under stress conditions, accumulation of proline in plants results either

from increased expression of proline synthetic enzymes or due to repressed activity of proline degradation (Hong et al. 2000). In our case, the increase in proline accumulation was greater in AS-2002 and lowest in SH-2002. Genotypic differences in proline accumulation under high temperatures were previously reported in 20 wheat genotypes (Ahmed and Hassan 2011). Leaf proline level is thought to serve as an effective index to screen wheat genotypes for relative differences in heat tolerance.

In the present study, increase in total leaf protein under heat stress was observed at both reproductive growth stages. It seems likely that this increase in total soluble proteins under heat stress is due to the induction of stress proteins, as such stress induced protein expression has been shown to be an important adaptive strategy of crop plants. Further, the majority of stress induced proteins is soluble in water and therefore contributes to stress tolerance presumably via hydration of cellular structures (Wahid and Close 2007). In our study, increased protein concentration resulting from heat treatments was similar in all the genotypes. Likewise, Din et al. (2011) observed no differences in leaf soluble protein concentration in various canola cultivars under water stress. Further studies are needed to determine if other wheat genotypes show variation for total leaf protein content as a response to heat stress.

Increased solute leakage is an indication of decreased cell membrane thermostability, and has long been used as an indirect measure of heat-stress tolerance in diverse plant species, including wheat (Blum et al. 2001, Wahid et al. 2007). In the present investigation, high temperature decreased the MSI at both growth stages in all the tested wheat genotypes. At anthesis, the decrease in MSI was greater in SH-2002 (23%) and lowest in AS-2002 (11%) and Inqalab-91 (15%). A similar trend of decreased MSI was exhibited by all the genotypes at milky growth stage. Yildirim et al. (2009) and Dhanda and Munjal (2006) concluded from their findings that membrane thermal stability was a useful selection criterion for heat stress tolerance in wheat, reporting differences in MSI among different wheat cultivars at various growth stages. As in that study, they also showed that the MSI of genotypes decreased towards later developmental stages, i.e. the milky stage. Membrane instability at the milky growth stage might be associated with the beginning of senescence. SIKDER et al. (2001) found significant correlation between membrane stability of flag leaf and grain yield, and suggested that membrane thermostability can be used to determine the heat tolerance of wheat varieties under heat stress conditions.

Heat stress is a common constraint during anthesis and grain filling stages in many cereal crops in both arid and temperate regions. At the reproductive phases, fertilization has been shown to be highly sensitive to high temperatures in various plants, whereas heat stress during wheat grain filling is known to reduce kernel growth and cause a reduction in kernel density and weight (Foolad 2005, Laghari et al. 2012) In the present study, heat treatment significantly increased the number of sterile florets per plant in all the tested wheat genotypes at anthesis growth stages. At anthesis, the highest number of sterile florets per spike after heat treatment occurred in Inqalab-91 (33.33), and the lowest in NR-234 (15.33) (Tab. 4). These results indicate that the genotypes examined here utilize unique responses for heat tolerance, as the lines showing the best performance as measured in proline, total protein, and MSI response show different heat responses regarding sterility and other heat associated traits.

Heat treatment decreased the grain yield per plant in all the tested wheat genotypes at both reproductive growth stages; however the most significant decrease was observed by

heat treatments during anthesis. There were significant variations in grain yield per plant within the genotypes both at anthesis and milky growth stages. At anthesis, the highest decease in grain yield was observed in Wafaq-2001 (80.5%), NR-234 (80.0%) and Punjab-96 (78.3%), and the least effect was observed for AS-2002 (62.9%). Khan and Hussain (2006) tested AS-2002 for heat tolerance in field studies, and found it had the best performance with respect to grain yield, followed by Inqalab-91. At the milky stage, the same cultivar also exhibited the least effect of heat stress on grain yield. Comparable studies show similar genotypic effects of heat on grain yield for wheat and other crop species (Khan et al. 2007, Nahar et al. 2010, Balouchi 2011). During the onset of meiosis in the male generative tissues until completion of anthesis, wheat grain setting is reduced by rises in temperature above optimum (Ferris et al. 1998). Our results are consistent with these previous reports, showing the heat effects on yield for new genotypes in wheat.

This study also shows that heat stress significantly decreases seed number and weight per spike in these wheat genotypes, more during the anthesis growth stage than the milky stage. For heat stress applied during anthesis, the relative seed number reduction was lowest in Punjab-96 followed by AS-2002 and Inqalab-91, and highest in NR-234 followed closely by SH-2002 and Wafaq-2001. Similarly, for anthesis applied heat stress, the seed weight per spike was greater in Inqalab-91 and AS-2002, and lowest in NR-234. For heat stress applied during the milky stage, the seed number and weight per spike was reduced, but the effect was small and not significant. In the present study, high temperature treatment at anthesis stage increased the number of sterile florets in all the tested genotypes, and most notably in the Inqalab-91, consequently reducing the number of grains per plant and the yield. Genotypic variation in grain yield for heat effects on the number of sterile florets was also observed. Among the reproductive stages, fertilization (1–3 days after anthesis) is one of the most sensitive to high temperatures (Foolad 2005). Pollen viability, patterns of assimilates partitioning, and growth and development of seed/grain, ear or spike are likewise highly affected by heat. Cereal crops can tolerate only narrow temperature ranges, and if these are exceeded during the flowering stages they can damage fertilization and seed set, resulting in yield reduction (Porter 2005). It has been shown for example that supra-optimal temperatures during grain filling decreased wheat yield by reducing kernel weight (Gibson and Paulsen 1999). In the current project, the decline in grain yield due to heat treatment at the milky stage is due to reduced seed weight per spike. In wheat, genotypic variation with respect to translocation of assimilates from source to sink under high temperature has already been reported (Mohammadi et al. 2009). The high grain yield and seed weight of the AS-2002 and Inqalab-91 cultivars may be due to their greater efficiency in mobilizing reserves from leaves, stem or other plant parts towards sink (Gupta et al. 2011). Furthermore, high temperatures can reduce the number of grains per spike, by causing either flower sterility or seed abortion, which reduces grain yield and ultimately harvest index causing smaller grain yield. Wardlaw (2002) found reductions in grain weight due to high temperature during both anthesis and milky growth stages under field conditions.

Conclusion

Tolerance to heat stress is a complex phenomenon and is controlled by multiple genes imparting a number of physiological and biochemical changes. No single trait fully explains why some wheat varieties are able to generate better yield under heat stress. Wide

variation in tolerance to heat stress existed in the tested wheat genotypes. Thus, there is a dire need for the development of heat tolerant wheat genotypes through a breeding program for cultivation under heat stress environments like those prevailing in Pakistan. The cumulative response of AS-2002 was better on the basis of physiological and yield attributes, indicating AS-2002 may represent an excellent parental material for a breeding program. Plant breeders should also use proline and MSI as selection markers in the breeding program for development of heat tolerant wheat cultivars, as these were shown here to be closely associated with yield in wheat.

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